Supplementary Online Content

Lindner D, Fitzek A, Bräuninger H, et al. Association of cardiac infection with SARS-CoV-2 in confirmed COVID-19 autopsy cases. *JAMA Cardiol*. Published online July 27, 2020. doi:10.1001/jamacardio.2020.3551

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Gene expression analysis

To isolate total RNA, snap frozen cardiac tissue was added to QIAzol and disrupted in the tissue lyser II. RNA was further purified using the miRNeasy mini kit (Qiagen, Germany) according to the manufacturer's protocol. To avoid genomic DNA contamination, DNase I (Qiagen, Germany) was applied directly on the column during the purification protocol, as suggested. The resulting RNA concentration was determined using the Nanodrop 2000c spectrophotometer and RNA was stored at -80 °C for further processing. After reverse transcription, cDNA was further diluted to a final working concentration of 10 ng/µl.

Virus load and replication were calculated as copy numbers using a quantified PCR product as standard. To assess gene expression for target genes, real-time PCR was performed using 2.5 µl gene expression master mix (Thermo Fisher Scientific, USA) and 0.25 µl specific gene expression assay. Gene expression assays listed in eTable 1 include forward and reverse primers as well the FAM-labelled probe. As template 1 µl of cDNA was used in a final volume of 5 µl. Real-time PCR was carried out on a QuantStudio 7 system (Applied Biosystems, USA). Each sample was analyzed in duplicates. Furthermore, the gene expression of *CDKN1B* was determined as endogenous control to normalize the data using the formula $2^{-\Delta Ct}$ and plotted as heat map as x-fold to *CDKN1B* using Morpheus (https://software.broadinstitute.org/morpheus).

Histological analysis

Cardiac specimens were fixed in 4 % formalin for approximately 48h and subsequently dehydrated and embedded in paraffin. Four micrometer-thick sections were cut and deparaffinised using xylene substitute (Rothihistol, Roth) and ethanol.

Cross sections were stained using hematoxylin and eosin (RO/T865.2, Roth) to visualize infiltrates. In addition, immunohistochemical staining using antibodies directed against $CD3^+$, $CD45R0^+$ and $CD68^+$ cells were performed and quantified as previously described.[1] Antibodies are listed in eTable 2. Staining were quantified by digital image analysis.[2] Images were taken using a Keyence BX-9000 microscope.

eTable 1. Gene expression assays were purchased from Thermo Fisher Scientific

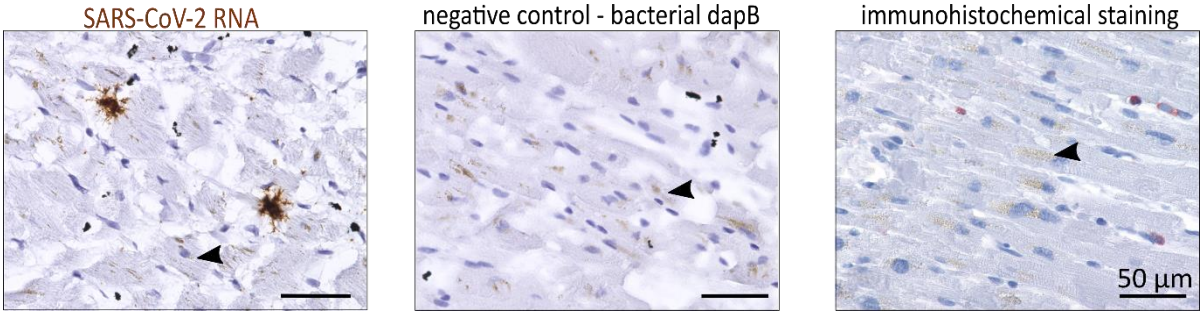
Gene name	Gene symbol	Assay ID
Tumor necrosis factor alpha	*TNF-α*	Hs00174128_m1
Interferon gamma	*IFN-γ*	Hs00989291_m1
C-C motif chemokine ligand 5	*CCL5*	Hs00982282_m1
Interleukin 6	*IL6*	Hs00174131_m1
Interleukin 8 / C-X-C motif chemokine ligand 8	*IL8 / CXCL8*	Hs00174103_m1
Interleukin 18	*IL18*	Hs01038788_m1
Cyclin dependent kinase inhibitor 1B	*CDKN1b*	Hs00153277_m1

eTable 2. Primary antibodies used for immunohistological analyses

Target	Dilution	Company	Ordering information
CD3	1:25	Agilent	M7254
CD68	1:100	Agilent	M0876
CD45R0	1:300	Agilent	M074201

eFigure. In situ hybridization - RNAScope

Viral SARS-Cov-2 RNA was detected via RNAScope on paraffin sections, while negative control probe against bacterial dihydrodipicolinate reductase (DapB, #310043) revealed no staining. Arrows indicate lipofuscin background, visible in negative control as well as in immunhistochemical staining as shown in the Figure.



As a negative control, probes detecting the bacterial dihydro-dipicolinate reductase (DapB) was used and revealed no specific staining. Lipofuscin is indicated by arrows and found frequently due to the elderly patient cohort.

eReferences

1. Escher F, Pietsch H, Aleshcheva G, et al. Detection of viral SARS-CoV-2 genomes and histopathological changes in endomyocardial biopsies. *ESC Heart Fail.* 2020.
2. Schultheiss HP, Fairweather D, Caforio ALP, et al. Dilated cardiomyopathy. *Nat Rev Dis Primers.* 2019;5(1):32.